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Short communication

Determination of three nitroimidazole residues in poultry meat by gas chromatography with nitrogen–phosphorus detection

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Abstract

A method was developed for the determination of the nitroimidazole compounds dimetridazole (DMZ), ronidazole (RNZ) and metronidazole (MNZ) by gas chromatography with nitrogen phosphorus detection. Nitroimidazole compounds were extracted with acetonitrile, followed by acidification using acetic acid and cleanup using strong cation-exchange (SCX) SPE column. Validation in chicken muscle fortified at a concentration of 5 μg/kg gave mean recoveries of 85% DMZ, 90% RNZ, 80% MNZ with RSDs of 13.0, 14.3, 11.2%, respectively ($n=6$). The method is suitable for statutory residue testing and is used as a quick screening method in the National Residue Surveillance Plan in China. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Food analysis; Dimetridazole; Ronidazole; Metronidazole

1. Introduction

Dimetridazole (DMZ), ronidazole (RNZ) and metronidazole (MNZ) are nitroimidazole compounds used primarily to prevent and treat histomoniasis and coccidiosis in poultry. These compounds are suspected carcinogens and mutagens. Their use in food producing species is prohibited within the EU. The decision was made for RNZ in 1993 [1], for DMZ in 1995 [2] and for MNZ in 1998 [3]. In China, RNZ, MNZ and DMZ have not been allowed to use in food-producing animals since 1999 in accordance with the circular of the Chinese Ministry of Agriculture.

Methods have been published for the determination of nitroimidazole at trace levels in biological matrices, but most of them qualifying only DMZ and its metabolite 2-hydroxymethyl-1-methyl-5-nitroimidazole (DMZOH). These methods mainly consisted of liquid chromatography — ultraviolet detection [4–6] or electrochemical detection [7]. Only a few methods have been published for the determination of multiple nitroimidazole residues.

Aerts et al. [8] developed a method for the determination of DMZ, RNZ, ipronidazole and their metabolites in poultry tissue and eggs using HPLC–UV. Gaugain et al. [9] reported a TLC method for the detection of RNZ, DMZ and DMZOH in animal muscle. Semeniuk et al. [10] described a HPLC–UV method for the determination of DMZ, MNZ in poultry tissue, serum and eggs.

In recent years, LC–MS has been applied for the determination of nitroimidazole residues in animal tissue [11–13]. LC–MS is the preferred technique

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for confirmatory residue analyses, however, it is not available in many laboratories.

Gas chromatographic analysis was carried out after esterification for the determination of DMZ in meat by GC–ECD [14], which require a tedious esterification stage.

Until now, only one reference was found to determine DMZ, RNZ and MNZ residues simultaneously in animal products [13].

Because gas chromatography with nitrogen–phosphorus detection (GC–NPD) possess higher sensitivity and selectivity for nitroimidazole compounds than other detection techniques, in this paper a new multi-residue screening method is developed and described for the determination of DMZ, RNZ and MNZ in chicken meat without derivatization directly using GC–NPD.

2. Experimental

2.1. Chemicals and reagents

Acetone, acetonitrile (MeCN), methanol (MeOH) were of HPLC reagent grade (Merck, Darmstadt, Germany). Other chemicals were of analytical reagent grade and made in China. DMZ, RNZ and MNZ: standard stock solutions (0.5 mg/ml) in MeOH were prepared monthly and kept frozen in colored vials. Spiking solution was made from standard stock solutions. Triphenylphosphate (TPP) was used as an internal standard (IS) and prepared in methanol. Working mixture standard solutions were prepared daily by properly diluting stock solutions and adding I.S. SPE cartridges (Bond-Elut SCX 500 mg/2.8 ml) were obtained from Varian (Harbor City, CA, USA).

2.2. GC–NPD equipment and chromatographic conditions

A Hewlett-Packard model 5890 series II gas chromatograph equipped with a nitrogen–phosphorus detector (NPD), a split-splitless injector connected to a HP3398A GC ChemStation A.01.01 (Hewlett-Packard, Palo Alto, CA) was used. The capillary column was HP Ultra-2 (5% diphenyl, 95% dimethylpolysiloxane) (25 m×0.32 mm I.D., 0.52 μm film thickness). The injector and detector was operated at 250 and 300°C, respectively.

The sample (2 ml) was injected in the splitless mode and purge valve was on at 1.0 min. The oven temperature was programmed as follows: 80°C for 1 min, rising to 173°C at a rate 25°C/min, to 185°C at a rate of 2°C/min, and finally to 260 at 30°C/min, held for 3 min. Gas flow-rate: nitrogen carrier gas, 1.5 ml/min; nitrogen makeup gas, 30 ml/min; hydrogen (H_2), 4 ml/min; and air, 120 ml/min.

2.3. Sample preparation

The extraction and clean-up procedure was based on the method described by Sams et al. [12]. The method was modified slightly. Tissue (5 g) was extracted twice by homogenization with MeCN (2× 25 ml) and then sodium sulfate (5 g) was added and mixed by swirling. The extractant was centrifuged (2400 *g* for 5 min) each time, then filtered through Na_2SO_4 and collected in a disposable plastic tube. Acetic acid (5 ml) was added and the contents were agitated with a vortex mixer. A Bond-Elut strong cation-exchange (SCX) solid-phase extraction (SPE) cartridge fitted with a 25-ml Varian reservoir was preconditioned by addition of MeCN–acetic acid (95:5, v/v; 5 ml). The above filtrate was transferred to the reservoir and allowed to run through the SPE column at a flow of 1 ml/min under vacuum. The cartridge was washed with acetone (2.5 ml) methanol (5 ml) and acetonitrile (5 ml) in sequence. The residue acetonitrile was removed under vacuum for 5 min and nitroimidazole were eluated into a 15-ml disposable plastic tube by the MeCN–28% ammonia solution (95:5, v/v; 5 ml). After addition of diethylene glycol–methanol (10:90, v/v; 100 μl), the eluate was evaporated to dryness on a model CNM-MS-1 multi-function sample treatment instrument for microchemical method (Changsha Zhongxun Electronic Engineering Institute, Changsha, China) under nitrogen stream at 35°C. The residues were reconstituted in 1 ml methanol containing 80 ppb TPP for GC analysis. All calculations were performed using internal standard method.

3. Results and discussion

GC–NPD proved to be suitable for the determination of nitroimidazole because of the specific response to these volatility nitrogen-containing compounds. Use of an I.S. is indispensable for accurate determination because the reproducibility of injection with I.S. was better than that without I.S. Identification can also carried out by retention time relative to I.S. On the basis of the TPP retention time in relation to residue and matrix components the compound proved to be acceptable as an internal standard.

The nitroimidazole residues are usually extracted from meat with an organic solvent such as methylene chloride and MeCN and further clean up is made by liquid–liquid partition with pH adjustment [4,5,8] or solid-phase extraction (SPE) with silica [6,11] and C_{18} [10] bonded phase cartridges. But some interfering matrix peaks were present at the same retention times as nitroimidazoles using these methods in the GC–NPD chromatogram.

It was found by experiment that the method described by Sams et al. [12] can provide cleaner solution, avoid the use of the chlorinated solvent and improve extraction efficiency. The basic procedures are listed as follows: Nitroimidazoles form cations at acidic condition. The protonated analyte is retained on SCX column by ionic interaction with benzenesulfonic acid group. The ionic interactions are much stronger than the non-polar interactions, allowing the use of extensive wash sequence and giving cleaner extracts. Elution is achieved by addition of ammoniacal MeCN, which deprotonates the analytes and breaks the ionic interaction. The final eluants evaporation step is critical. The use of a small volume of low volatility solvent ethylene glycol (boiling point 241°C) as a ''keeper'' and not allowing the evaporating temperature to exceed 40°C help to improve the recovery. Because nitroimidazoles were easily absorbed onto the glassware, we used plastic tubes during all operation. Nitroimidazole are very light sensitive, therefore, all experimental procedures were performed in a dim room.

The calibration curves from the peak area ratio of standards and I.S. to concentration of standards were obtained. Each calibration curve had shown acceptable linearity correlation coefficients of $r>0.998$ at concentrations ranging from 10 to 200 ng/ml, which correspond to the residues content in the range of 2–40 ng/g in 5-g sample.

Table 1
Recovery data for chicken muscle fortified at 5 and 10 μg/kg ($n=6$)

Analyte	Spiked concentration (μg/kg)	Mean recovery (%)	RSD (%)
DMZ	5	85	13.0
	10	91	14.1
RNZ	5	90	14.3
	10	82	10.0
MNZ	5	80	11.2
	10	78	13.2

Limits of detection, based on signal three the baseline noise of blank matrix, were 0.2 ng/g for DMZ, MNZ and 0.5 ng/g for RNZ.

Recovery and repeatability data were obtained by analysis of control samples fortified at an appropriate concentration for chicken muscle down to 5 ng/g. The results are given in Table 1.

Fig. 1a shows the chromatogram of a blank chicken muscle. Fig. 1b shows the chromatogram of a blank chicken muscle spiked with nitroimidazoles at a concentration of 10 μg/kg.

4. Conclusion

The GC–NPD method described in the paper provides a simple, rapid and reliable procedure for quantitative analysis of nitroimidazoles in animal tissues. The method can be used to detect nitroimidazole residues in chicken muscle to ensure that the current MRL of 10 μg/kg is not exceeded. Suspected samples found by the proposed method were further analyzed and confirmed with LC–MS or GC–MS. The method is suitable for statutory residue testing and is used as a quick screening method in the National Residue Surveillance Plan in China.

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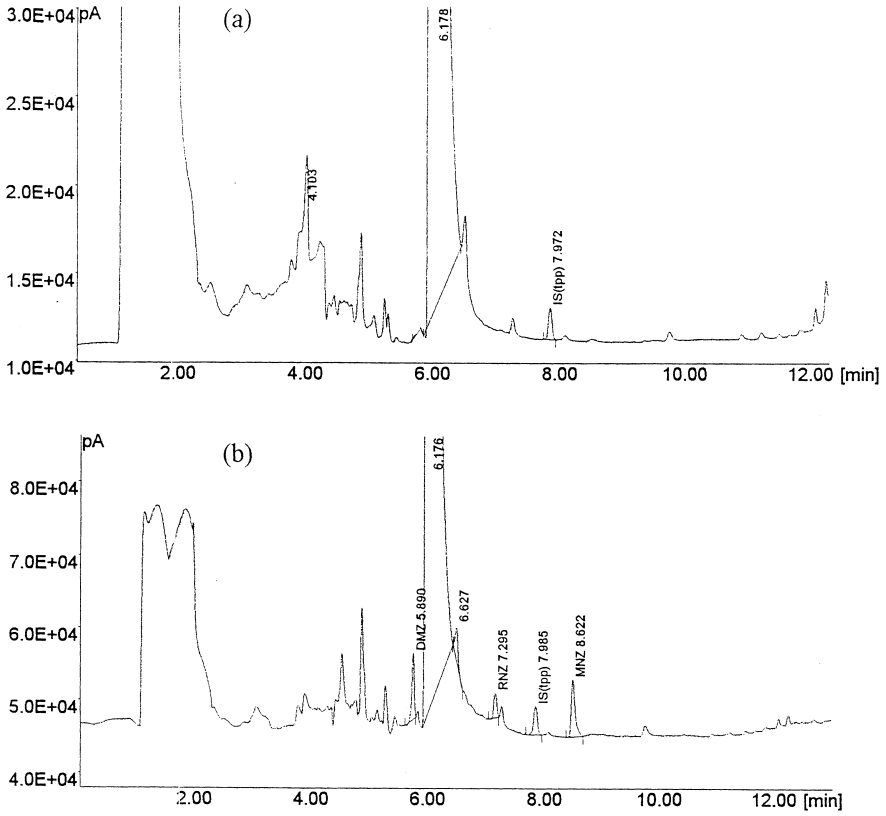

Fig. 1. GC–NPD chromatograms of chicken muscle (a) blank, (b) same blank spiked with DMZ, RNZ, MNZ at a concentration of 10 μg/kg.

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